The methoxymethyl cation cleaves peptide bonds in the gas phase†

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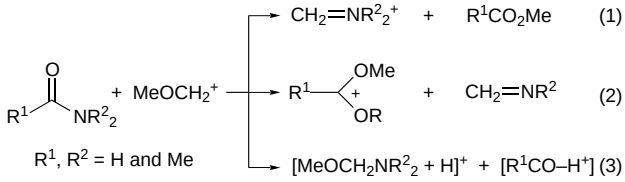
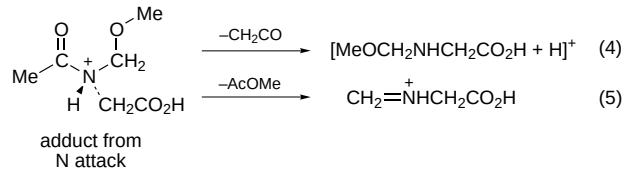
Methoxymethyl cations and simple *N*-acyl amino acids and dipeptides react in the gas phase to form $[M + MeOCH_2]^+$ ions which fragment *via* a number of pathways including amide bond cleavage.

A major goal in modern mass spectrometry is the development of techniques to structurally characterize molecules of biological significance. As part of our ongoing research into the gas phase electrophilic modification of biomolecules and model compounds,[1,2] we became interested in designing reagents to sequence biomolecules *via* specific *gas phase* ion–molecule reactions. Thus for peptides, the ultimate aim is to develop site specific ion–molecule reactions to cleave the peptide bonds in an analogous fashion to solution phase chemistry such as the Edman technique.[3–5] Although at first glance such an effort may seem ambitious, we were encouraged during a recent flowing afterglow study on the rates of ion–molecule reactions of the methoxymethyl cation. In particular, we rediscovered a reaction[6] which leads to products arising from amide bond cleavage [eqns. (1)–(3)].

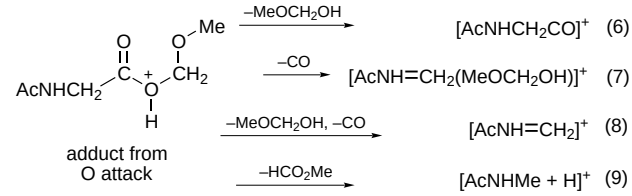


Here we present preliminary results designed to address the question: can the methoxymethyl cation be used to cleave peptide bonds in the gas phase? Experiments were performed on a VG ZAB-2HF reverse sector tandem mass spectrometer.[7] Methoxymethyl cations, which were generated in the chemical ionization source using $MeOCH_2CH_2OMe$ as the CI gas, were allowed to react with peptides and model systems introduced *via* a direct insertion probe. Typical source conditions were: source temperature = 250 °C; electron energy = 70 eV; emission current = 200 mA; source pressure = 7×10^{-4} Torr, measured on the source ion gauge. In each case, three major alkylated product ions $[M + MeOCH_2]^+$, $[M + Me]^+$ and $[M + CH]^+$ were formed, consistent with previous studies.[2b,6] In order to gain some insights into the regioselectivity of attack by $MeOCH_2^+$, collisional activated mass-analysed ion kinetic energy (CA-MIKE) spectra were carried out on each of the $[M + MeOCH_2]^+$ ions using argon as the collision gas (at a pressure of 2×10^{-6} Torr).

The first model systems we chose to study were the *N*-acyl derivatives of glycine and alanine (Table 1).‡ The $[M + MeOCH_2]^+$ ions of these systems both fragment *via* loss of CH_2CO and $MeCO_2CH_3$. These losses are best rationalized as occuring *via* attack at the amide nitrogen atom to give a nitrogen adduct which then undergoes amide bond cleavage as shown in eqn. (4) [*c.f.* eqn. (3)] and eqn. (5) [*c.f.* eqn. (1)]. Further



products due to loss of $MeOCH_2OH$, $[MeOCH_2OH + CO]$ and HCO_2Me (this loss might arise from hydride ion transfer followed by a proton transfer[8]) are also observed which may arise from initial attack at the C terminus to give an oxygen adduct which then undergoes the fragmentations shown in eqns. (6)–(9). Thus the methoxymethyl cation not only cleaves amide



bonds, but also cleaves the C–OH bond of the carboxylic acid group.

We next turned our attention to the $[M + MeOCH_2]^+$ ions of the free dipeptides glycylglycine, glycylalanine and alanylglycine, which all only fragment to form $[M + CH]^+$ ions as the major product with $[M + Me]^+$ ion formation as a minor reaction channel (Table 1). The lack of attack by the methoxymethyl

Table 1 CID MS/MS Spectra of $[M + MeOCH_2]^+$ ions of peptides and model systems

Neutral species (*m/z* of $[M + MeOCH_2]^+$)	Daughter ions (intensity)[a]
$MeC(O)NHCH_2CO_2H$ (162)	132(54), 130(100), 120(2.5), 100(2.5), 88(5), 74(5), 72(17), 45(1)
$MeC(O)^{15}NHCH_2CO_2H$ (163)	133(15), 131(100), 121(2), 101(1.5), 89(3.5), 75(2), 73(5), 45(1)
$MeC(O)NHCD_2CO_2H$ (164)	134(21), 130(100), 122(3), 102(2), 90(5), 76(5), 74(9), 45(1)
$MeC(O)NHCHMeCO_2H$ (176)	146(24), 144(100), 134(1.5), 114(16), 102(11), 86(4), 45(*)
$H_2NCH_2C(O)NHCH_2CO_2H$ (177)	147(2), 145(100)
$H_2NCH_2C(O)NHCHMeCO_2H$ (191)	161(2), 159(100)
$H_2NCHMeC(O)NHCH_2CO_2H$ (191)	161(3), 159(100)
$MeC(O)NHCH_2C(O)NHCH_2CO_2H$ (219)	201(4), 189(6), 187(100), 176(*), 157(*), 145(2), 120(1), 88(1)

[a] (*) Designates an ion with an intensity of less than 1.

cation onto the peptide bond with concomitant fragmentation is consistent with the known gas phase reactivity of glycine[2a] as well as the basicity of the various functional groups within dipeptides. For example, *ab initio* calculations have shown the following basicity order for glycylglycine: N-terminal amino group > oxygen of amide group > nitrogen of amide group > carboxyl carbonyl oxygen).[9]

In order to make the peptide bonds in these dipeptides more susceptible to attack by the $MeOCH_2^+$ ion, we have examined the effects of moderating the gas phase reactivity of the N-terminal amino group *via N*-acetylation. Thus the $[M + MeOCH_2]^+$ ions of *N*-acetyl derivatives‡ of glycylglycine, glycylalanine and alanylglycine not only decompose to form $[M + CH]^+$ and $[M + Me]^+$ ions, but more importantly, several other product ions resulting from amide bond cleavage were observed (Table 1 and Fig. 1). In particular, the CA-MIKE spectra of the $[M + MeOCH_2]^+$ ions of these *N*-acetyl derivatives indicate that attack by the methoxymethyl cation onto the heteroatom adjacent to a C=O group followed by bond cleavage are general. Thus attack at all three sites (labelled as 1–3 in Fig. 2) occurs, thereby providing *complete* sequence information. Consequently distinction between *N*-acetylglycylalanine and *N*-acetylalanylglycine is readily made (Fig. 1). Interestingly, cleavage of the peptide bonds in these instances gives rise to fragments ions (Fig. 2) where the charge is retained at the C terminus (*i.e.* modified Y ions such as $[Y_n + CH]^+$ and $[Y_1 + CH_3OCH_2]^+$ are formed), while the only fragment ion in which the charge is retained at the N terminus is that due to cleavage of the C terminus OH bond to give a B_3 ion [for sequence ion nomenclature, see ref. 4(*a*)]. Thus not only does the methoxymethyl cation cleave the peptide bonds, but it cleaves them in a specific fashion, thereby simplifying the mass spectra.

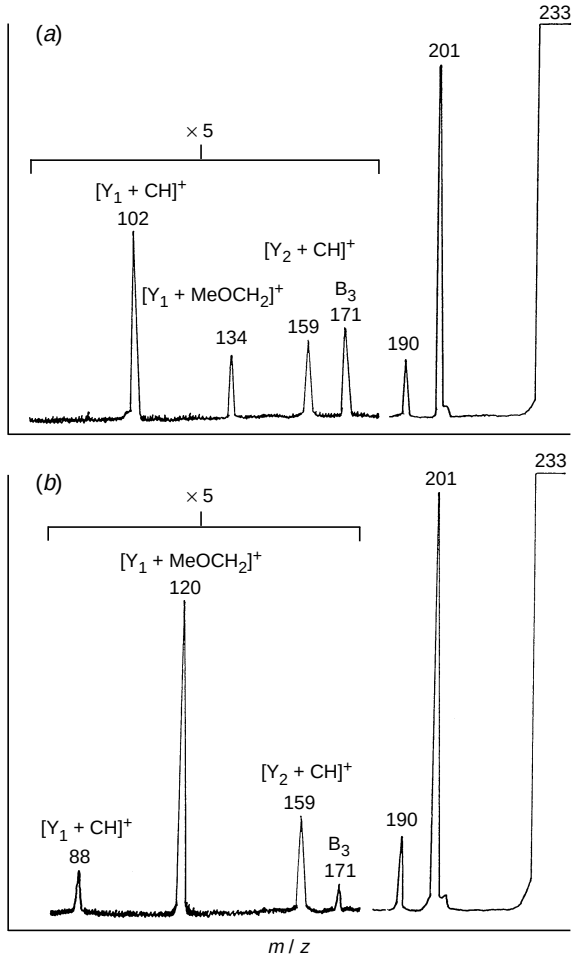


Fig. 1 CA-MIKE spectra of the $[M + MeOCH_2]^+$ ions of (*a*) *N*-acetylglycylalanine and (*b*) *N*-acetylalanylglycine

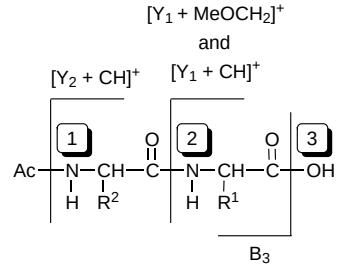


Fig. 2

Further work is required to determine the general utility of these reactions to sequence small peptides in the gas phase *via* cleavage of their peptide bonds (the inherent limitations of volatility of the peptide could potentially be overcome using laser desorption CI[10]). In particular the effects of residues with potentially reactive side chains (*e.g.* lysine, aspartic acid *etc*.) need to be investigated. Future studies will also examine the modes of reactivity of other reagent ions such as $ClCH_2^+$.

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Footnotes

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‡ *N*-Acetyl derivatives were prepared *via* a modified literature procedure (D. P. Knapp, *Methods Enzymol.*, 1990, **193**, 314): The α-amino acid or dipeptide (0.002 mol) was dissolved in 2 ml of 2 M NaOH and chilled in an ice bath. Upon complete dropwise addition of acetic anhydride (2 ml), the mixture was stirred for a further 10 min followed by careful acidification to pH < 4 with H_2SO_4. The solution was allowed to warm to room temperature and extracted with EtOAc (3 × 25 ml). The combined EtOAc layers were dried with anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The resultant *N*-acetyl derivatives were purified *via* recrystallization from MeOH. All other compounds were of reagent grade obtained commercially and were used without further purification. $H_2{}^{15}NCH_2CO_2H$ (99 atom% ^{15}N) and $H_2NCD_2CO_2H$ (98 atom% D) were both obtained from Cambridge Isotope Laboratories.

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